CONCENTRATIONS AND DISTRIBUTIONS OF PERFLUORINATED COMPOUNDS IN HUMAN SERUM FROM REPRESENTATIVE GENERAL POPULATION, KOREA

Kim HY[1], Kim SK[2], Kang DM[3,4], Hwang YS[4], Oh JE[1*]

[1]Department of Civil and Environmental Engineering, Pusan National University, Jangjeon-dong, Geumjeong-gu, Busan 609-735, Korea; [2]Department of Marine Science College of Natural Science, Incheon University, Songdo-dong, Yeonsu-gu, Incheon 406-772, Korea; [3]Department of Preventive and Occupational Medicine, School of Medicine, Pusan National University, Yangsan, Gyeongnam, 626-870, Korea; [4]Environmental Health Center for Asbestos, Pusan National University Yangsan Hospital, Yangsan, Gyeongnam, 626-770, Korea

Introduction

According to the industry development and quality of life improvement, the usage of chemical products rapidly increased. Among these chemicals, the perfluorinated compounds (PFCs) that have both, hydrophobic and oleophobic characteristic draw an attention these days due to the designation of 'New-POPs' by the Stockholm convention. Since 1960, PFCs have been widely used in commercial and industrial products such as food packaging, fire-fighting foams, leather, carpets, paper coatings and semiconductor (Zushi et al., 2008) resulting from their unique properties.

Despite of these advantages of PFCs, the PFCs are not degraded well by hydrolysis, photolysis and bio-degradation etc in the environmental matrices, thus have been detected in all environmental compartments as well as human serum and food (Tittlemier et al., 2007; Fromme et al., 2009). These days, in several toxicological studies on PFCs, the adverse health effects are reported that PFOS and PFOA may disturb fatty acid metabolism and affect the reproductive system and induce adverse effect on liver and other tissues (Seacat et al., 2002; Kennedy et al., 2004). For this reason, many studies have been conducted to investigate PFCs exposure concentration in human body (Kannan et al., 2004; Kato et al., 2011). In Korea, several PFCs exposure studies for high exposure group (ex, manufacturing workers) and sensitive populations (ex, mother) were performed (Chung et al., 2008; Jang et al., 2008; Kim et al., 2011) but there is limited information on general population. Therefore, the purposes of this study were to investigate the concentration of PFCs in the general Korean population and to understand relationship between the PFCs concentrations and age, gender and region.

Materials and methods

Chemicals and Materials

Potassium perfluorobutane sulfonate (PFBS), sodium perfluorohexane sulfonate (PFHxS), Sodium perfluoroheptane sulfonate (PFHpS), Sodium perfluorooctane sulfonate (PFOS), Sodium perfluorodecane sulfonate (PFDS), perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUdA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluoro-n-[$^{13}C_4$]butanoic acid (MPFBA), perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid (MPFHxA) ,perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid (MPFOA), perfluoro-n-[1,2-$^{13}C_2$]decanoic acid (MPFDA), perfluoro-n-[1,2-$^{13}C_2$]undecanoic acid (MPFUdA), perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid (MPFDoA), sodium perfluoro-1-hexane[$^{18}O_2$]sulfonate (MPFHxS), sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octane sulfonate were purchased from Wellington Laboratories (Guelph, ON, Canada). All standard solutions were diluted in HPLC grade methanol (J.T. Baker, USA) and stored in a polypropylene vials at 3-5 ℃. Ammonium acetate and formic acid were purchased from Wako and ammonium hydroxide was obtained from Junsei. Acetonitrile, Milli-Q water, methyl-*tert*-butyl ether (MTBE) were purchased from J.T Baker.

Sample collection and preparation

In our study, we investigated 16 PFCs concentrations in serum of adults over the age of 20 years in Busan metropolitan city, Korea. The proportionate stratified sampling method was used to collect serum samples that can represent the general Korean population and about 25~30 participants per one site were selected according to gender and the serum samples were collected by home visit. The 10 sites were selected for this research but one site of Pusan National University was added additionally to meet the ratio of age, 20-30 years. The total of 124 serum of male and 183 of female were collected from 11 sites and the detailed information was shown in Table 1. All serum samples were collected from volunteers and approved by Pusan National University Institutional Review Board (IRB).

During the sampling, participants in this study answered the questionnaire to provide basic information such as age, gender, living environment, eating habits, drinking habits, smoking habits and residential history. To reduce the response error, one-to-one survey was conducted a series of questions and answers. Approximately 5-6 mL of blood was collected from each donor and samples were centrifuged to separate serum in the field at 4000rpm for 10 mins. The serum samples were stored at −20℃ until analysis. In this study, 16 PFCs were analyzed in serum. The samples were extracted using solid-phase extraction (SPE) manifold system (Supelco; PA, USA).

Tabel 1. Infromation of participants in this study

Study site	Code No.	No. of samples							Age(years)
		Male	Female	20'	30'	40'	50'	≤60	Mean(range)
Dong-gu	C21	10	16	3	1	5	7	10	52 (22-68)
Dongrae-gu	C22	9	17	3	2	8	9	4	47 (25-67)
Haeundae-gu 1	C23	8	14	5	8	2	2	5	42 (22-70)
Haeundae-gu 2	C24	7	21	3	2	2	6	15	56 (21-69)
Saha-gu	C25	12	16	1	2	4	9	12	54 (28-67)
Geumjeong-gu	C26	10	15	3	2	4	9	7	51 (24-68)
Yeonje-gu	C27	11	18	2	4	11	5	7	49 (23-70)
Sasang-gu	C28	12	15	2	2	4	13	6	51 (20-63)
Busanjin-gu	C29	10	16	1	1	22	2	0	43 (20-57)
Suyeong-gu	C30	8	17	2	2	6	7	8	50 (21-71)
Additional	C31	27	18	26	18	1	0	0	28 (20-40)
Total		124	183	51	44	69	69	74	46 (20-71)

Instrumental analysis

The identification of 16 PFCs in samples was accomplished with an Agilent 1200 high-performance liquid chromatography (HPLC) system coupled with and an Agilent 6460 electrospray triple-quadrupole mass spectrometer (ESI-MS-MS). Complex medium for the separation of PFCs in the serum was used for ZORBAX XDB-C18 column (4.6 x 150 mm, 5μm; Agilent, USA). The mobile phases for a binary gradient were methanol and 2mM ammonium acetate. The target compounds were identified and quantified using the multiple reaction monitoring (MRM) mode.

Quality Assurance and Control

The all standards were prepared with methanol and nine-point standard calibration curve with the concentration range from 0.05 to 50 ng/mL was drawn for every batch of analysis. The correlation coefficients (r^2) of the calibration curves were over 0.99. Limits of detection (LODs) were determined on the basis of a signal-to-noise ratio of 3 (S/N=3), which ranged from 0.13 to 0.76 ng/mL. The spiked recoveries of 16 PFCs were from 50 to 104 % (n=3). The blanks for sample pre-treatment procedure and instrument contamination were checked for every batch (n=20) of analysis, and the levels of blank were less than the LODs for all target compounds.

Statistical Analysis

The statistical analysis of data was performed using the SPSS 18.0K (Korean version; SPSS, Inc., Chicago, IL, USA). To evaluate the correlation between PFCs concentration and gender, age, we performed T-test and ANOVA test respectively. Statistical significance of these results was evaluated according to the p-value ($p<0.05$). PCA was conducted to assess the PFCs distribution patterns according to gender, age and region, .

Results and discussion

PFCs concentration in serum samples

14 of 16 target PFCs were identified and the concentration of total PFCs ranged from 5.87 to 91.7 ng/mL. PFOA and PFOS were dominant compounds with a fraction of 22% and 34% of the total PFCs, respectively. Similar to other previous results, PFOS was shown the highest concentrations (mean; 13.2 ng/mL), (Ericson et al., 2007; Christensen et al., 2011). Also PFHxS (mean; 2.61 ng/mL), PFOA (mean; 8.37 ng/mL), PFNA (mean; 2.92 ng/mL), PFDA (mean; 1.46 ng/mL) and PFUdA (mean; 2.00 ng/mL) were detected with more than 90% detection frequency.

Most of previous studies on human exposure to PFCs were focused on PFOS and PFOA so we compared these two PFCs concentrations with other countries. In this study, the level of PFOA (0.77~23.0 ng/mL) was a little bit higher or similar with those in USA (Olsen et al., 2007), Spain (Ericson et al., 2007), Japan (Miyake et al., 2007) and Austrailia (Ericson et al., 2007). When compared with previous 5 Korean studies, the PFOA concentration of this study was higher except two cases of target citizens of Daegu (Kannan et al., 2004) and workers in manufacture (Jang et al., 2008). For PFOS, the levels of this study (1.93~49.5 ng/mL) were similar or lower than other results (Miyake et al, 2007; Olsen et al., 2003). Compared with previous Korean studies, most results of PFOS were similar to ours except the one case of citizen of Daegu (Kannan (2004)).

Correlation between the PFCs concentrations and region, gender and age

The PFCs result of 11 study sites was shown in Fig. 1(a) and the total average concentration of 11 sites was 38.6 ng/mL. The lowest concentration was found in the C23 (mean; 30.3 ng/mL) and the highest concentration was found in the C24 (mean; 47.5 ng/mL) even though C23 and C24 sites are located just beside. The C23 is one of the wealthiest districts and C4 is the poor district in the city of Busan (XX). Recently, a study was reported that the social status could affect the exposure of chemicals like PFCs and BPA. They evaluated the relationship between diet, use of consumer products and other activities, behaviors or circumstances and PFCs in serum level, presenting the positive association of the PFC levels and family income (Nelson et al., 2012). We were not able to investigate the participants' individual income, but C23 and C22 are commonly known as the wealthy districts and C24 and C21are poor districts in Busan, thus there is a possibility of the effect of social status on PFCs exposure, which is needed further investigation.. Unlike other 8 general residential areas, C25 (mean; 40.1 ng/mL) and C28 (mean; 43.4 ng/mL) are located near the industrial area. So we expected the high PFCs levels in serum concentration of these two sites because industry was regarded as one of PFCs major sources (Yeung et al., 2006) but couldn't find significant difference compared to other sites. The PFCs concentration of 124 males and 183 females was shown in Figure 1(b). The male and female's average concentrations of total PFCs were 34.5 (7.49~91.7) and 30.1 (5.87~81.5) ng/mL, respectively and the total PFCs concentration of male was higher. All PFCs compounds concentration except PFHxA, PFBA, PFPeA, PFHpA and PFTrDA in male was higher than in female. However, when we conducted t-test with PFCs concentrations, the statistically significant difference was not found.

As shown in Figure 1(b), the PFCs concentration is increasing according to age regardless of sex. To verify this tendency, ANOVA test was conducted and the statistically significant different p-values ($p<0.05$) were obtained, indicating the higher PFCs accumulation over the age. However, because of missing values for the age under the twenty and over the age of seventy, further investigation is needed to confirm the PFCs trends with age.

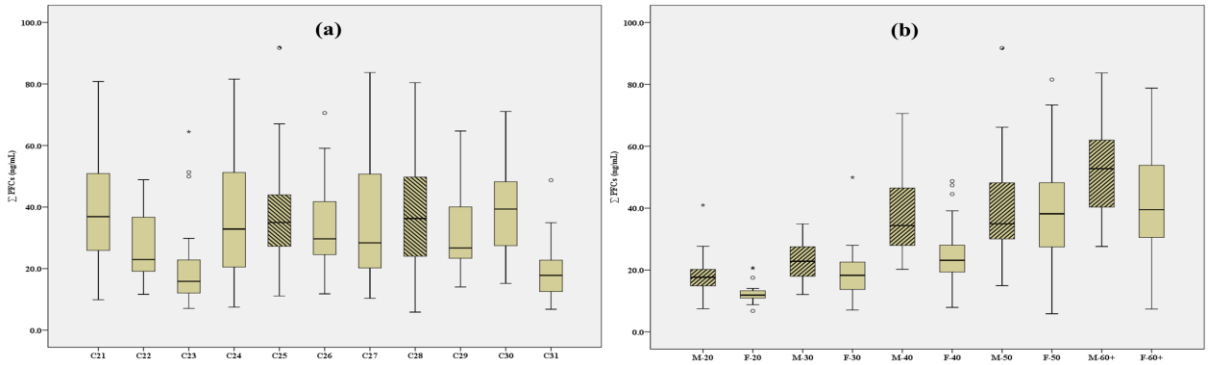

Fig. 1. PFCs concentration by region(a), gender and age(b).

Acknowledgements
This research was supported by a grant (11162KFDA725) from Korea Food & Drug Administration in 2011.

References:

1. Zushi Y., Takeda T. and Masunaga S. (2008). *Chemosphere.* 71(8): 1566-1573.
2. Tittlemier S. A., Pepper K., Seymour C., Moisey J., Bronson R., Cao X. L. and Dabeka R. W. (2007). *J Agric Food Chem.* 55(8): 3203-3210.
3. Fromme H., Tittlemier S. A., Völkel W., Wilhelm M. and Twardella D. (2009). *Int J Hyg Environ Health.* 212(3): 239-270.
4. Seacat A. M., Thomford P. J., Hansen K. J., Olsen G. W., Case M. T. and Butenhoff J. L. (2002). *Toxicological Sciences.* 68(1): 249-264.
5. Kennedy G. L., Butenhoff J. L., Olsen G. W., O'Connor J. C., Seacat A. M., Perkins R. G., Biegel L. B., Murphy S. R. and Farrar D. G. (2004). *Crit Rev Toxicol.* 34(4), 351-384.
6. Kannan K., Corsolini S., Falandysz J., Fillmann G., Kumar K. S., Loganathan B. G., Mohd M. A., Jesus Olivero O., Van Wouwe N. and Yang J. H. (2004). *Environ Sci Technol.* 38(17): 4489-4495.
7. Kato K., Wong L. Y., Jia L. T., Kuklenyik Z. and Calafat A. M. (2011). *Environ Sci Technol.* 45: 8031-8045.
8. Chung JY, Yoon HS, Ryu HY, Won JU, Paeng KU and Kim YJ. (2008). *Analytical Science & Technology*. 21(3): 183-190.
9. Jang SH, Lee CK, Kim DH, Kim KB, Ahn JH, Kim HD, Lee CH, Kim JH and Lee JT. Korean J Occup Environ Med. 20(3): 233-244.
10. Kim SK, Lee KT, Kang CS, Tao L., Kannan K., Kim KR, Kim CK, Lee JS, Park PS and Yoo YW. (2011). *Environmental Pollution.* 159(1): 169-174.
11. Ericson I., Gómez M., Nadal M., van Bavel B., Lindström G. and Domingo J. L. (2007). *Environ Int.* 33(5): 616-623.
12. Christensen K. Y., Maisonet M., Rubin C., Holmes A., Calafat A. M., Kato K., Flanders W. D., Heron J., McGeehin M. A. and Marcus M. (2011). *Environ Int.* 37(1): 129-135.
13. Olsen G. W., Burris J. M., Ehresman D. J., Froehlich J. W., Seacat A. M., Butenhoff J. L. and Zobel L. R. (2007). *Environ Health Perspect.* 115(9): 1298.
14. Miyake Y., Yamashita N., So M. K., Rostkowski P., Taniyasu S., Lam P. K. S. and Kannan K. (2007). *Journal of Chromatography A.* 1154(1-2): 214-221.
15. Olsen G. W., Church T. R., Miller J. P., Burris J. M., Hansen K. J., Lundberg J. K., Armitage J. B., Herron R. M., Medhdizadehkashi Z. and Nobiletti J. B. (2003). *Environ Health Perspect.* 111(16): 1892.
16. Nelson J. W., Hatch E. E. and Webster T. F. (2010). *Environ Health Perspect.* 118(2): 197-202.
17. Yeung L. W. Y., So M., Jiang G., Taniyasu S., Yamashita N., Song M., Wu Y., Li J., Giesy J. and Guruge K. (2006). *Environ Sci Technol.* 40(3): 715-720.